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IDH1/2 Mutations and BCL-2 Dependence: An Unexpected Chink in AML's Armour

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There is a pressing need to develop novel, mechanism-based therapeutic approaches that can be used to improve therapies for genetically defined tumor subtypes. Chan and colleagues have demonstrated recently that BCL-2 inhibitors can target *IDH1/2* mutant cancers through a mutant-specific dependency in metabolic regulation.

Our increasing knowledge of the somatic cancer genome has led to the development of molecularly targeted therapies for an ever-expanding list of cancer subtypes. This has included the development of tyrosine kinase inhibitors for hematologic and epithelial tumors, which have had a substantive clinical impact. However, there has been an increasing interest in the development of genotype-specific targeted therapies that do not target mutated proteins themselves, but rather leverage mutant-specific dependencies in a specific genomic context. In a recent issue of *Nature Medicine*, Chan et al. (2015) elucidate a critical role of *IDH1/2* mutations in deregulating mitochondrial function with therapeutic implications and suggest that BCL-2 inhibitors can target *IDH1/2* mutant cancers.

Isocitrate dehydrogenases (IDHs) catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) and NADPH during the citric acid cycle. Mutations in *IDH1* were first identified through exome sequencing in colorectal cancer (Sjöblom et al., 2006). Shortly thereafter, recurrent *IDH1* and *IDH2* mutations were found in glioma and acute myeloid leukemia (AML) (McKenney and Levine, 2013). The most common *IDH1* and *IDH2* mutations in AML affect arginine residues, R132 of IDH1 protein, or R140 and R172 of IDH2. Of note, metabolomics and biochemical studies have shown that the mutant enzymes gain a neomorphic activity that catalyzes the conversion of α-KG to (R)-2-hydroxyglutarate [(R)-2HG]. (R)-2HG may act as an oncometabolite by inhibiting the function of α-KG-dependent enzymes, including TET family enzymes, Jumonji-C domain containing proteins, and other critical regulators of the epigenetic state (Xu et al., 2011). As a consequence, these mutations can result in dysregulated angiogenesis, alterations in the pattern of histone modifications, and aberrant DNA methylation (Krell et al., 2013).

In addition to their impact on the epigenetic state, several studies have suggested that *IDH1/2* mutations can alter global cellular metabolism and that these alterations in metabolic milieu can contribute to oncogenic transformation. Reitman and Yan (2010) noted depletion of the tricarboxylic acid (TCA) cycle metabolites citrate, *cis*-aconitate, α-KG, malate, and fumarate and an accumulation of biosynthetic precursors in cell lines that expressed *IDH1* R132H and *IDH2* R172K mutations, suggesting that

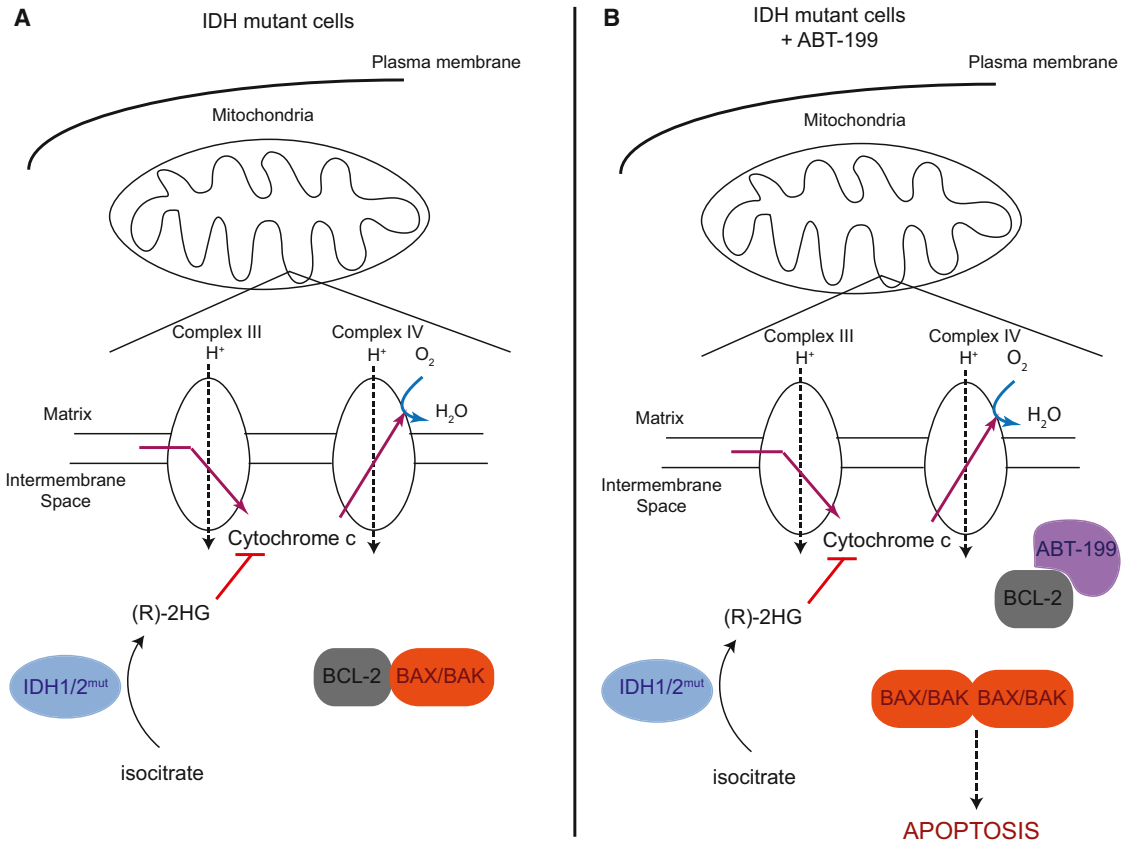

Figure 1. Synthetic Lethal Mechanism between *IDH1/2* Mutations and BCL-2: Definition of a New Therapeutic Target
(A) (R)-2HG, generated by *IDH1/2* mutants, inhibits cytochrome *c* release by complex IV (COX), which activates BAX/BAK signaling that is blocked by BCL-2 activity, allowing cell survival.
(B) ABT-199 treatment of *IDH1/2*-mutated cells permits full activation and oligomerization of BAX/BAK proteins, resulting in mitochondrial outer membrane permeabilization (MOMP)-induced apoptosis.

these mutations can result in TCA cycle downregulation. Furthermore, (R)-2HG may impair the mitochondrial respiratory chain through inhibition of cytochrome *c* oxidase and ATP synthase (Latini et al., 2005). However, the therapeutic relevance of these findings was not fully delineated.

Chan et al. (2015) performed a high content small hairpin RNA (shRNA) screen in isogenic leukemia cells expressing wild-type and mutant IDH1 and found that the survival of *IDH1/2* mutant cells was highly dependent on expression of the BCL-2 family members: BCL-2 and BCL-W (Chan et al., 2015). The authors then demonstrated a strong association between *IDH1/2* mutational status and BCL-2 family dependence in cell line systems and patient samples.

Most importantly, these observations suggested a near-term therapeutic opportunity. Anti-apoptotic BCL-2 family proteins antagonize death signaling by forming heterodimers with pro-death proteins, including BAX and BAK. Heterodimer formation occurs through binding of the pro-apoptotic protein's BH3 domain into the hydrophobic cleft of anti-apoptotic proteins. This insight led to the development of small molecule BH3 mimetics that function as competitive inhibitors of BCL-2 by binding to the hydrophobic cleft (Ni Chonghaile and Letai, 2008). As a highly specific BCL-2 inhibitor, ABT-199 is in late phase clinical trials in chronic lymphocytic leukemia and other lymphoid neoplasms. The authors therefore investigated the sensitivity of *IDH1/2* mutant and wild-type AML cells to ABT-199. Consistent with their genetic data, AML cells expressing mutant *IDH1/2* were more sensitive to ABT-199 compared to wild-type cells. More importantly, studies using patient-derived xenografts confirmed the in vitro studies, because *IDH1/2* mutant AML cells were sensitive to BCL-2 inhibition with ABT-199 in vivo. They also noted an impact of ABT-199 therapy on secondary transplantation assays, suggesting that *IDH1/2* mutant leukemia stem cells were also sensitive to ABT-199. These data underscore the potential therapeutic relevance of their observation, which can be tested in the clinical context.

Next Chan et al. (2015) investigated the mechanism involved in *IDH1/2* mutations' dependency on BCL-2 family members in AML cells. They first demonstrated that culturing wild-type *IDH1/2* leukemic cell lines or primary AML blasts with octyl-(R)-2HG, a cell-permeable precursor of (R)-2HG, could confer BCL-2 dependence. These data suggested the neomorphic activity of mutant IDH1/2 proteins was responsible for conferring BCL-2 dependence in AML cells. They then elucidated how *IDH1/2* mutations altered the balance between pro-apoptotic and anti-apoptotic members'

expression. In homeostatic conditions, BAX and BAK initiate apoptosis by causing mitochondrial outer membrane permeabilization, leading to cytochrome *c* release in the cytosol and collapse of the mitochondrial transmembrane potential ($\Delta\Psi_{mito}$) (Souers et al., 2013). The authors used the membrane-permeant JC-1 dye as an indicator of mitochondrial membrane potential and found that ABT-199 treatment of *IDH1/2* mutant cells or cells exposed to octyl-(R)-2HG resulted in a collapse of $\Delta\Psi_{mito}$. The effect of 2HG was enantiomer specific, because treatment with (S)-2HG had no impact on the time of mitochondrial transmembrane potential collapse. Mitochondrial studies showed that (R)-2HG had a specific effect on the activity of complex IV (also known as COX) enzymatic activity. Previous studies described that D-2-hydroxyglutaric acid (DGA) could inhibit complex IV activity and, to a lesser extent, complex V activity in muscular tissues (Latini et al., 2005). Consistent with these studies, the authors found that COX activity is also decreased in primary *IDH1/2* mutant cells without any change in the mitochondrial mass present in the cells.

Consistent with their in vitro and preclinical models, *IDH1/2* mutations, through (R)-2HG accumulation, may promote oncogenesis through their effect on mitochondrial respiration and promote dependence on BCL-2 for survival (Figure 1A). The observation that *IDH1/2* mutations confer sensitivity to ABT-199 may serve as eligibility criteria for ABT-199 trials in *IDH1/2* mutant leukemias and other *IDH1/2* mutant tumor types (Figure 1B). Moreover, these findings may have relevance for cancers without *IDH1/2* mutations. Specifically, the authors demonstrated that COX inhibitors decrease mitochondrial respiration and promote sensitivity to ABT-199, suggesting the possibility of combined electron transport chain and BCL-2 inhibition as a therapeutic approach in cancer. Moreover, this study highlighted the importance of mitochondrial dysfunction in this AML subtype and will lead to subsequent studies of metabolic vulnerabilities in AML and other cancers.

Most importantly, these studies provide a strong rationale for investigating the efficacy of BCL-2 inhibition in *IDH1/2* mutant cancer and add to the expanding armamentarium of targeted cancer therapies for this genetically defined cancer subset. Taken together, these discoveries provide new hope for patients with *IDH* mutant malignancies and provide a clarion example of how functional studies can be used to nominate novel, genotype-specific therapies.

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